SUPPLEMENTARY INFORMATION

ER residency of the ceramide phosphoethanolamine synthase SMSr relies on homotypic oligomerization mediated by its SAM domain

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SUPPLEMENTARY METHODS

Synthesis of photoactivatable and clickable lipid analogues

A 15 carbon-long fatty acid containing a photo-activatable diazerine and clickable alkyne group, pacFA, was synthesized in 3 steps from commercially available educts as described in Haberkant *et al*[1]. Next, pacFA was coupled to D-*erythro*-sphingosine (Enzo Biochem) using a combination of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) and hydroxybenzotriazole (HOBT) as condensing reagents, yielding the photo-activatable and clickable C15-ceramide analogue, pacCer (85% overall yield). pacPC was synthesized starting from 1-oleoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (Avanti Polar Lipids) and pacFA under the action of N,N-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) with satisfactory yield (39%). pacDAG was synthesied in 3 steps starting from 1-oleoyl-*sn*-glycerol (Santa Cruz Biotechnology). First, the primary HO-group was protected with the triphenylmethyl protecting group (trityl-chloride/pyridine; 92% overall yield). The glycerol obtained was coupled with the pacFA using EDCI/DMAP activation (58% overall yield). The final deprotection step was achieved using trifluoroacetic acid (TFAA) to generate pacDAG (28% overall yield). pacPE was synthesied in 3 steps starting from 1-oleoyl-2-hydroxy-*sn*-glycero-3-phosphoethanolamine (Avanti Polar Lipids). First, the amino-group was protected with the *tert*-butoxycarbonyl protecting group (di-*tert*-bytuldicarbonate/triethylamine; 98% overall yield). The ethanolamine obtained was coupled with pacFA using EDCI/DMAP activation in a good yield (52%). The final deprotection step was achieved with TFAA to generate pacPE (35%, overall yield). pacSM was synthesized starting from sphingosylphosphorylcholine (lyso-SM d18:1, Avanti Polar Lipids) and pacFA under the action of EDCI/HOBT (78% overall yield). pacCPE was synthesized in a single step from pacSM using a transphosphatidylation exchange reaction of choline fragment to ethanolamine initiated by phospholipase D Type VII from *Streptomyces sp*. (Sigma Aldrich), essentially as described in Fletcher *et al*[2]. All synthetic compounds were purified by thin layer chromatography to a high degree (purity >98%) and their structures were confirmed by 1H and 13C NMR and electrospray-ionisation mass spectrometry (ESI MS). The synthetis of photoactivatable and clickable lipid analogous will be described in detail in (S. Bockelmann, S. Korneev, J. Mina, P. Haberkant and J. Holthuis, manuscript in preparation).

Generation of SMSr$^{-/-}$ cells

Generation of a CRISPR/Cas9-mediated SMSr-knockout HeLa cell line (HeLa SMSr$^{-/-}$) was performed essentially as described Mali *et al.*[3]. Briefly, a gBlock containing the CRISPR target sequence was designed as follows:

cacagtcagacagtgactcaGTGTCACAgctagcTTTCCCATGATTCCTTCATATTTGCATATACGATACAAG
GCTGTTAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGA
CGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATA
TGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACA
CCG**TCAACTCTGCATTCGCCGC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGT

TATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTggatccTGTGCACAgtcagtcacagtcagtcta
c - (CRISPR target sequence for SMSr in bold and underlined).

The gBlock was digested with NheI and BamHI and ligated into the NheI/BamHI sites of a CMV promoter-deleted pCDH-EF1-Hygro vector (re-named pCDH-CMV(-); SBI; CD515B-1). HeLa cells stably transfected with doxycycline-inducible Cas9 expression construct pCW-Cas9 (kindly provided by F.G. Tafesse, Oregon Health and Science University, Portland, Oregon) were transfected with pCDH-CMV(-)SMSr/sgRNA and cultured in media containing 7μg/ml puromycin and 250μg/ml hygromycin B (Invitrogen). Stably transfected cells were treated with 2μg/ml of doxycycline (Sigma-Aldrich) for 3-5 days. The cells were transferred to a 96-well plate at clonal dilution. Individual colonies were picked, and propagated. SMSr−/− cells were selected by immunoblotting using an affinity-purified rabbit polyclonal anti-SMSr antibody[4] and CPE synthase activity assay as described in Vacaru *et al*.[5].

REFERENCES

1. Haberkant, P. *et al.* In vivo profiling and visualization of cellular protein-lipid interactions using bifunctional fatty acids. *Angew. Chem. Int. Ed. Engl.* **52,** 4033–8 (2013).
2. Fletcher, S., Ahmad, A., Price, W. S., Jorgensen, M. R. & Miller, A. D. Biophysical properties of CDAN/DOPE-analogue lipoplexes account for enhanced gene delivery. *ChemBioChem* **9,** 455–463 (2008).
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5. Vacaru, A. M. *et al.* Sphingomyelin synthase-related protein SMSr controls ceramide homeostasis in the ER. *J. Cell Biol.* **185,** 1013–27 (2009).

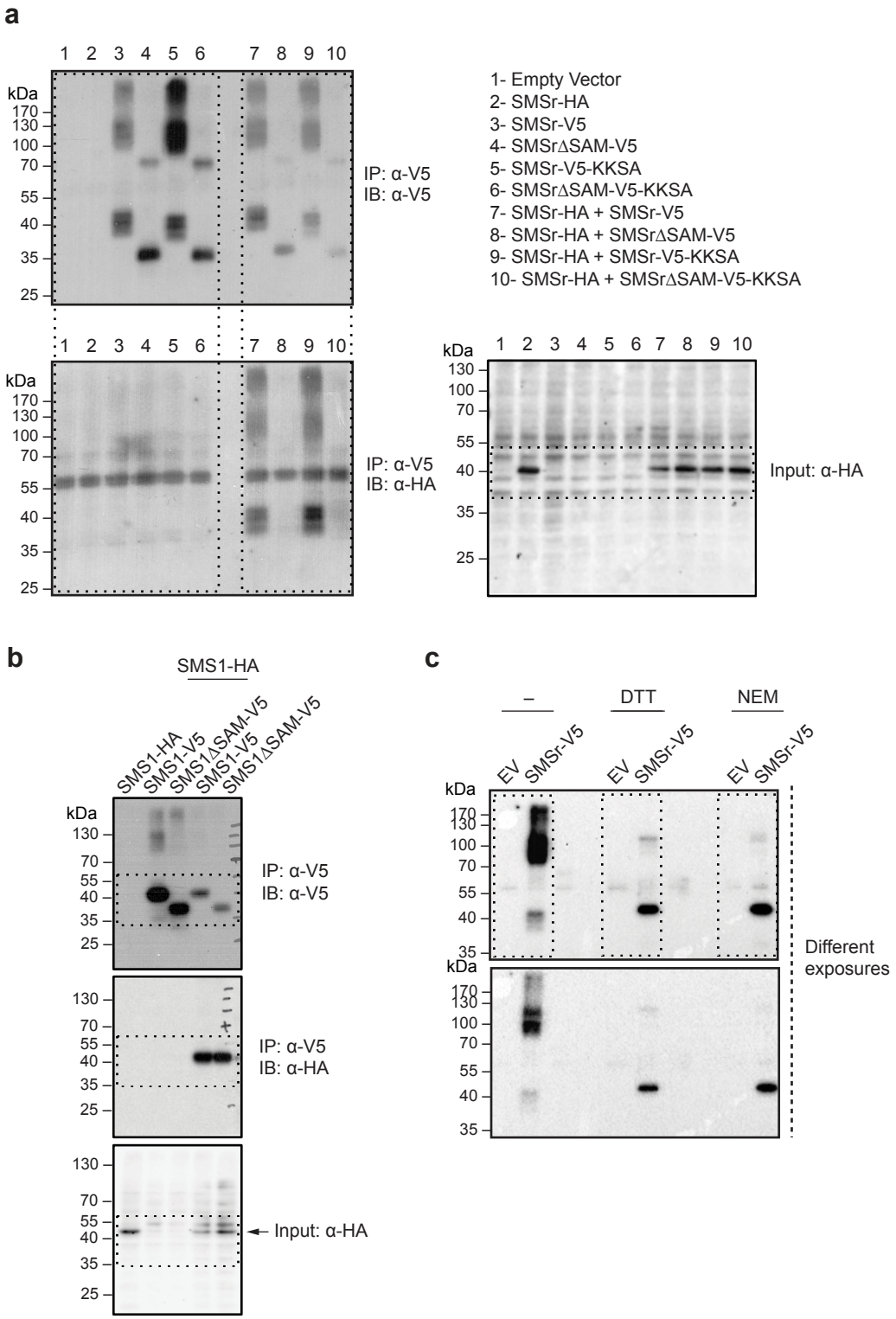

Figure S1. Uncropped blots shown in Figure 3. Uncropped images of blots used to make the corresponding panels in Fig. 3 are shown. Only parts of the blots delineated by dashed boxes were used. Other annotations are as in Fig. 3 and are described in its legend.

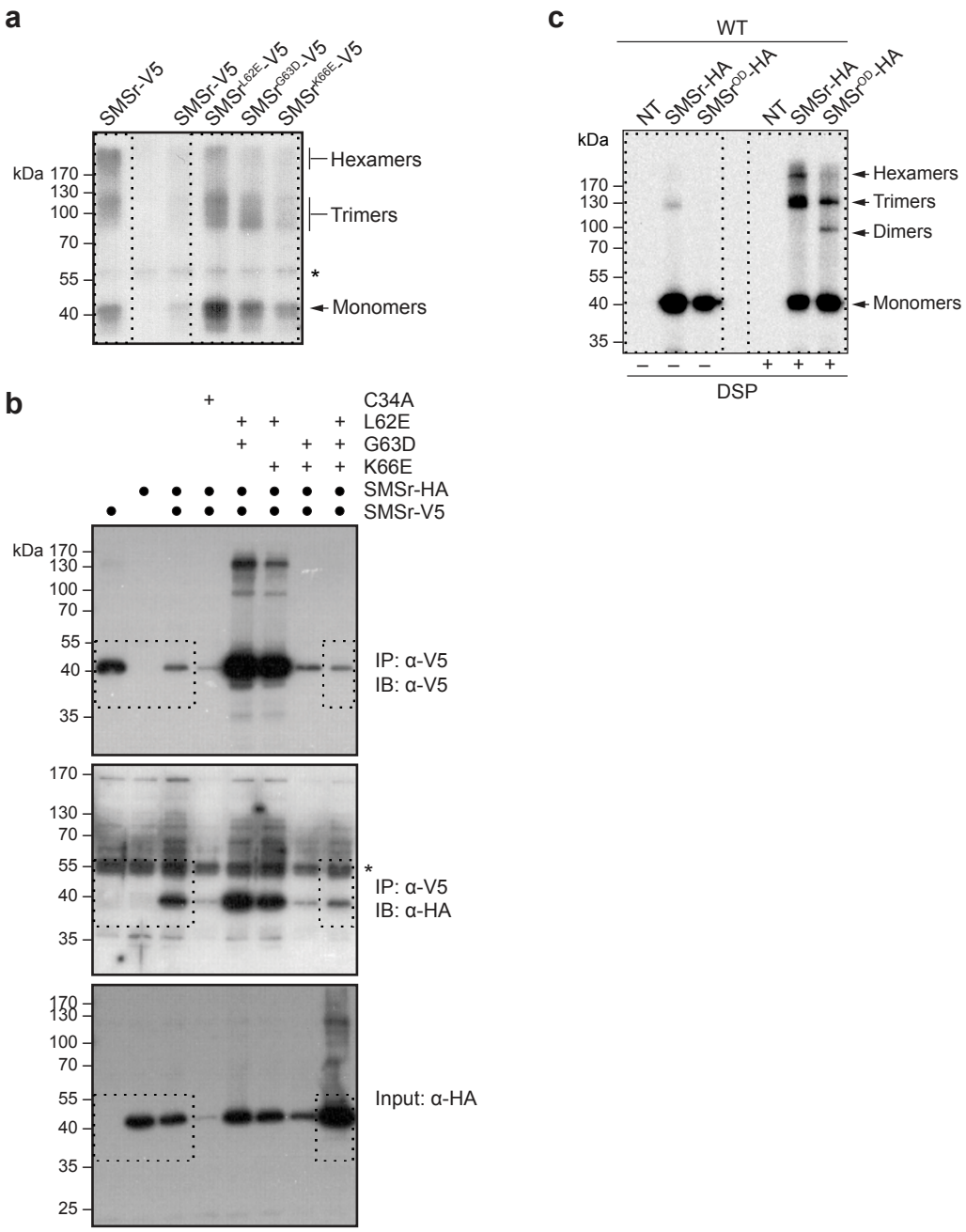

Figure S2. Uncropped blots shown in Figure 4. Uncropped images of blots used to make the corresponding panels in Fig. 4 are shown. Only parts of the blots delineated by dashed boxes were used. Other annotations are as in Fig. 4 and are described in its legend.

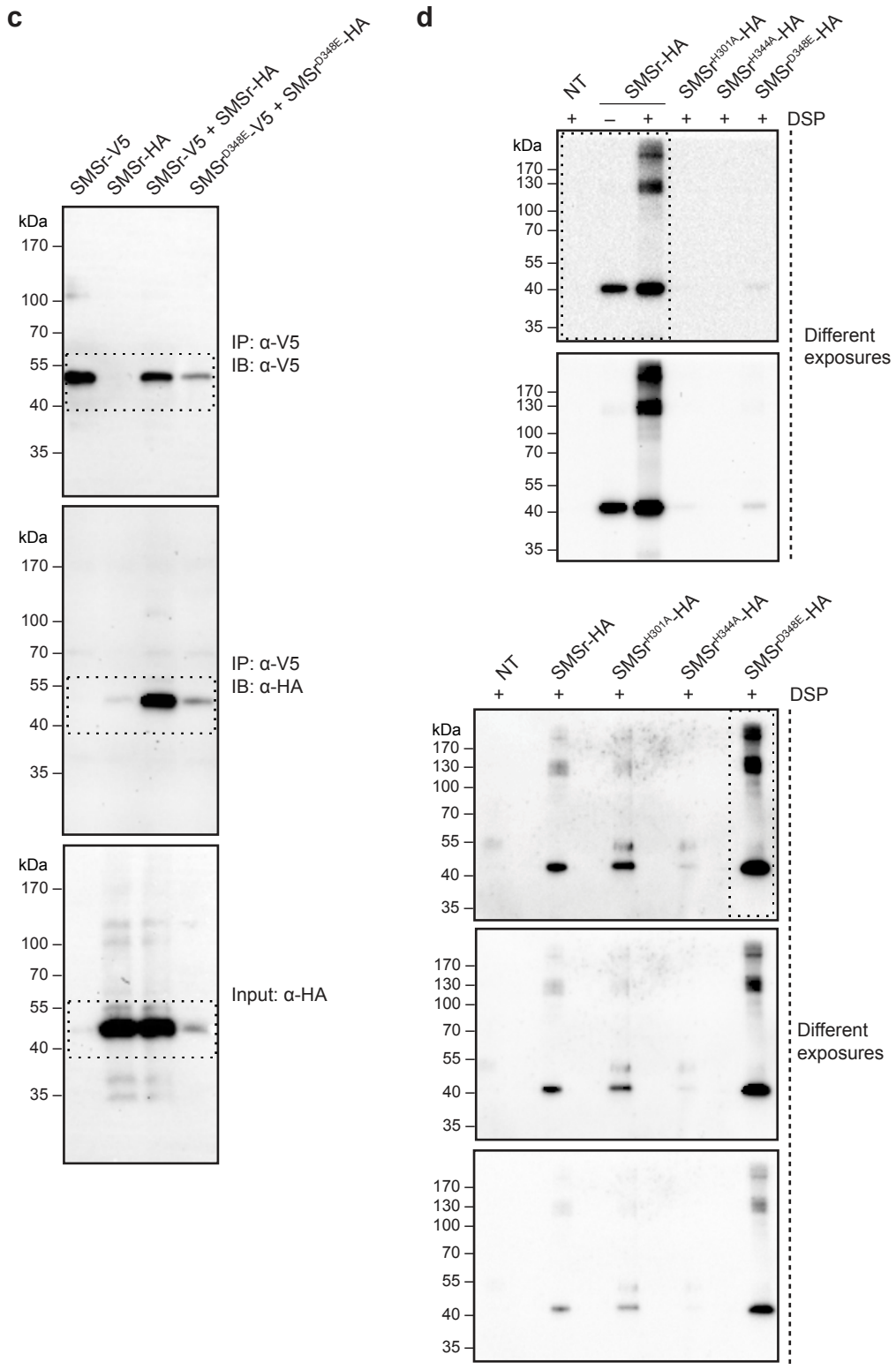

Figure S3. Uncropped blots shown in Figure 5. Uncropped images of blots used to make the corresponding panels in Fig. 5 are shown. Only parts of the blots delineated by dashed boxes were used. Other annotations are as in Fig. 5 and are described in its legend.

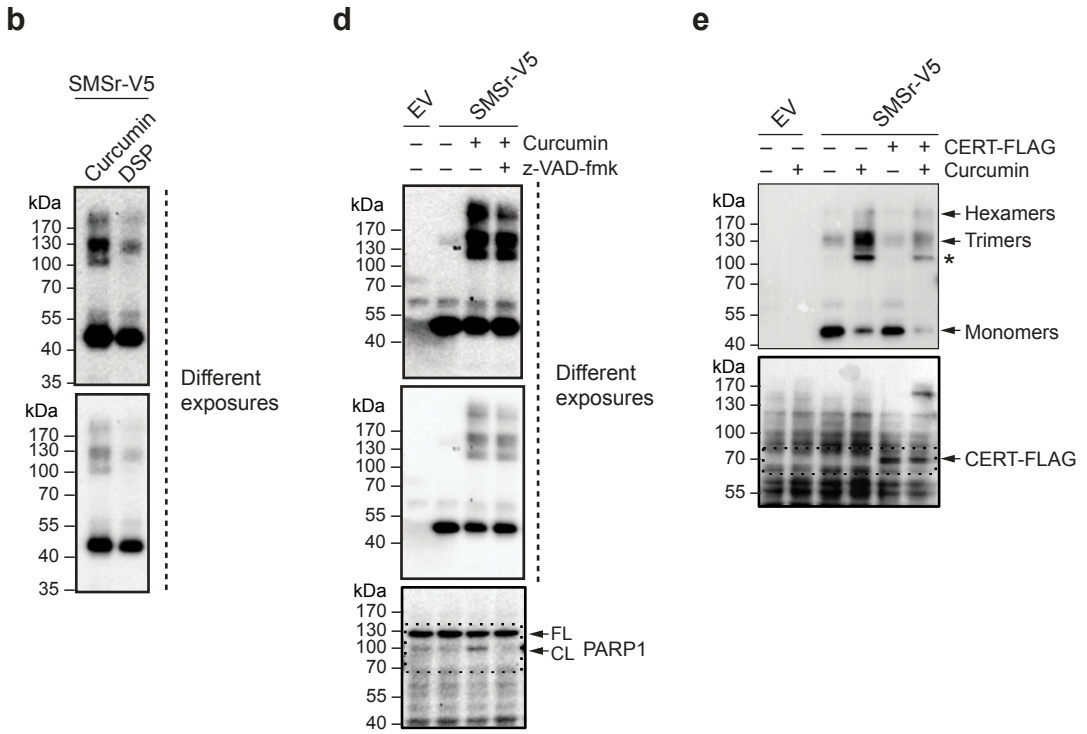

Figure S4. Uncropped blots shown in Figure 6. Uncropped images of blots used to make the corresponding panels in Fig. 6 are shown. Only parts of the blots delineated by dashed boxes were used. Other annotations are as in Fig. 6 and are described in its legend.

Query: hSMSr-SAM					
Accession	*Description*	*Query cover*	*E value*	*Ident*	*Accession*
SAMD8	Sphingomyelin synthase-related protein 1	100%	2E-40	100%	Q96LT4.2
DGKD	Diacylglycerol kinase delta	98%	3.E-05	33%	Q16760.4
DGKH	Diacylglycerol kinase eta	98%	1.E-04	35%	Q86XP1.1
LIPB2	Liprin-beta-2	95%	0.002	38%	Q8ND30.3
CSKI1	Caskin-1	100%	0.006	36%	Q8WXD9.1
SMS1	Sphingomyelin synthase 1	68%	0.02	37%	Q86VZ5.2
BICC1	Protein bicaudal C homolog 1	79%	0.049	32%	Q9H694.2
ETV6	Transcription factor ETV6	94%	0.1	35%	P41212.1
LIPB1	Liprin-beta-1	95%	0.27	34%	Q86W92.2
BFAR	Bifunctional apoptosis regulator	100%	0.58	25%	Q9NZS9.1

Query: hDGKd-SAM					
Accession	*Description*	*Query cover*	*E value*	*Ident*	*Accession*
DGKD	Diacylglycerol kinase delta	100%	9.E-40	100%	Q16760.4
DGKH	Diacylglycerol kinase eta	100%	1.E-30	83%	Q86XP1.1
SHAN2	SH3 and multiple ankyrin repeat domains protein 2	95%	7.E-12	48%	Q9UPX8.3
SHAN3	SH3 and multiple ankyrin repeat domains protein 3	100%	2.E-09	39%	Q9BYB0.3
SHAN1	SH3 and multiple ankyrin repeat domains protein 1	84%	3.E-09	48%	Q9Y566.2
CNKR3	Connector enhancer of kinase suppressor of ras 3	85%	1.E-07	37%	Q6P9H4.1
CNKR2	Connector enhancer of kinase suppressor of ras 2	85%	5.E-07	37%	Q8WXI2.1
SAM15	Sterile alpha motif domain-containing protein 15	95%	9.E-07	36%	Q9P1V8.1
BICC1	Protein bicaudal C homolog 1	81%	1.E-05	37%	Q9H694.2
SAMD8	Sphingomyelin synthase-related protein 1	98%	3.E-05	33%	Q96LT4.2

Query: hSMS1-SAM					
Accession	*Description*	*Query cover*	*E value*	*Ident*	*Accession*
SMS1	Sphingomyelin synthase 1	100%	2E-41	100%	Q86VZ5.2
UBIP1	Upstream-binding protein 1	81%	2.E-04	33%	Q9NZI7.1
SAMD8	Sphingomyelin synthase-related protein 1	67%	2.E-04	37%	Q96LT4.2
LIPB1	Liprin-beta-1	62%	6.E-04	31%	Q86W92.2
CNKR3	Connector enhancer of kinase suppressor of ras 3	73%	0.002	27%	Q6P9H4.1
TF2L1	Transcription factor CP2-like protein 1	90%	0.003	26%	Q9NZI6.1
TFCP2	Alpha-globin transcription factor CP2	56%	0.003	31%	Q12800.2
ICAM4	Intercellular adhesion molecule 4	42%	0.007	43%	Q14773.1
CNKR2	Connector enhancer of kinase suppressor of ras 2	71%	0.008	26%	Q8WXI2.1
SPDEF	SAM pointed domain-containing Ets transcription factor	62%	0.013	27%	O95238.1

Supplementary Table 1. BLAST search reveals a high level of similarity between DGKδ-SAM and SMSr-SAM. blastp searches (*http://blast.ncbi.nlm.nih.gov*) were performed in the swissprot database of human proteome. Queries sequences used are hSMSr-SAM, Q96LT4:12-78; hDGKδ-SAM, Q16760:1145-1208; hSMS1-SAM, Q86VZ5:13-76.

Query	Accession	Residue #
human SMSr-SAM	NP_001167627.1	12-78
mouse SMSr-SAM	NP_080559.1	75-141
chicken SMSr-SAM	XP_426501.3	12-78
frog SMSr-SAM	NP_001016197.1	11-77
zebrafish SMSr-SAM	NP_001082939.1	12-78
human DGKδ-SAM	NP_690618.2	1141-1205
mouse DGKδ-SAM	NP_808314.2	1147-1211
chicken DGKδ-SAM	XP_422569.4	1096-1160
frog DGKδ-SAM	XP_004917921.1	1117-1181
zebrafish DGKη-SAM	XP_005168036.1	1186-1249
human SMS1-SAM	NP_671512.1	1-72
mouse SMS1-SAM	NP_659041.3	7-78
chicken SMS1-SAM	NP_989721.2	1-72
frog SMS1-SAM	NP_001008197.1	1-72
zebrafish SMS1-SAM	NP_001071082.1	1-72

Supplementary Table 2. Accession numbers and amino acid residues used to create the phylogenetic tree in Figure 2c